APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1982, p. 179–183
0099-2240/82/070179-05$02.00/0
Vol. 44, No. 1

Comparison of Methods for Quantitative Determinations of Airborne Bacteria and Evaluation of Total Viable Counts

I. MONICA LUNDHOLM†

Departments of Environmental Hygiene and Clinical Bacteriology, University of Gothenburg, Sweden

Received 9 June 1981/Accepted 8 March 1982

Three different methods of estimating airborne bacteria were compared. An Andersen sampler, a slit sampler, an impinger, and filter samplers with gelatine filters or membrane filters were tested for collection efficiency. The comparisons were made in laboratory experiments with an aerosol of *Staphylococcus epidermidis* or *Serratia marcescens*, in field experiments in two different industries, i.e., cotton mill and sewage plant, and in experiments with skin fragment sampling. Experiments were also performed estimating the total number of viable microorganisms on the airborne particles. The Andersen sampler gave the highest bacterial counts in all environments tested. The slit sampler gave statistically lower counts only in the aerosol experiments and cotton mill experiments, where the size of the majority of the particles carrying visible bacteria was 2 to 6 μm or smaller. In the sewage plant and skin fragment experiments, where the particles were mainly 5 μm or larger, the difference was not significant. The filters were efficient in sampling in skin fragment experiments only. With the agar impingement method, the total viable cell count showed a rising index value with increasing particle size. A mean of 13 bacteria was found per particle in the cotton mill, a mean of 24 in the sewage plant, and a mean of 147 in skin fragment experiments.

Though airborne microorganisms are found in almost all environments, they do not normally present a health hazard to the exposed individual. In certain environments, such as hospitals, airborne infection is considered important and has been thoroughly investigated (4). A number of industrial settings such as cotton mills and sewage plants (5, 6, 20) may contain pathogenic microorganisms or high concentrations of normal contaminants which may represent a health risk. These environments have not yet been investigated to the same extent. Methods for measuring the number of airborne organisms are thus of general hygienic interest.

Airborne bacteria may be collected with a variety of different samplers and grown in the laboratory to calculate the total number of organisms present in the studied atmosphere. The different physical principles of the samplers may also affect their sampling efficiency of different species of bacteria. When bacteria or bacterial aggregates impinge on an agar surface, one colony appears after incubation. Although the number of such colonies is often used to express the number of airborne viable bacteria, the true total number of viable microorganisms can only be obtained after the airborne aggregates of bacteria have been dispersed (12, 17).

The aim of the investigation reported here was to test some commercially available samplers used to determine quantities of airborne bacteria and to evaluate their suitability for industrial environments contaminated with different kinds of microorganisms. Samplers were tested for collection efficiency in laboratory experiments, in skin fragment sampling, and under field conditions in different industries.

Experiments estimating the total number of viable microorganisms on the airborne particles in relation to the total viable particle number were performed in the different environments to determine the true exposure dose.

MATERIALS AND METHODS

Air samplers. (i) Impactor samplers. Two types were used, with standard plastic petri dishes. One was the Casella slit sampler, model Mk II (C. F. Casella & Co. Ltd., London) (2), with a sampling velocity of 30 liters/min, measured according to the manufacturer. The other was a six-stage Andersen sampler (1) with a flow rate of 28.3 liters/min. The volume of agar in the petri dishes was adjusted for maximal collection efficiency (1), and the agar plates were counted by the "positive hole" method on all stages (1, 13).

(ii) Liquid impinger sampler. The all-glass impinger (Greenburg-Smith impinger) was used with a sampling capacity of 11.5 liters/min. The collecting fluid was a

† Present address: Department of Clinical Microbiology, University Hospital, S-751 22 Uppsala, Sweden.

phosphate buffer (phosphate-buffered distilled water, pH 7.2). The sampling fluid was stored at +4°C and was quantitated in less than 4 h. The impinger fluid was filtered through a 0.45-μm cellulose membrane (Millipore Corp.) filter, which was placed directly onto an agar plate.

(iii) Filtration samplers. Two types of filters were used: gelatin filters (Sartorius, Göttingen, West Germany), with a pore size of 3 μm and a flow rate of 15 liters/min, and cellulose membrane filters (Millipore Corp., Bedford, Mass.), with a pore size of 0.45 μm and a flow rate of 15 or 17 liters/min. After sampling, the filters were placed on agar plates where the gelatin filters dissolved.

Flow rate and sampling time. The air flow through the samplers, with the exception of the slit sampler, was in all experiments verified with a rotameter. No extra inlet tubes were used on the samplers.

Bacterial media. Blood agar plates were used as a nonselective medium, and Drigalski agar was used as the selective medium for gram-negative rods. Plates were incubated at 35 or 30°C (sewage plant samples) and were counted after about 40 h.

Bacterial strains sampled. (i) Aerosol experiments. The bacteria studied in the exposure chamber experiments were *Staphylococcus epidermidis* (subgroup VI, NCTC 7944) and a biochemically active, pigmented *Serratia marcescens* isolated at the Department of Clinical Bacteriology, Gothenburg, Sweden. The bacteria were harvested from an 18-h broth culture and diluted in physiological saline to a concentration of about 10^4 bacteria per ml.

(ii) Field experiments. Experiments were run in cotton mills and in a sewage plant; in these experiments, only gram-negative rods were sampled. Bacteria in cotton mills become airborne during the processing of raw cotton, which contains bacteria mainly originating from the cotton plant (21). Sewage water contains primarily gram-negative rods, which become aerosolized when the sewage water is sprinkled or agitated.

(iii) Skin fragment experiments. Experiments with airborne skin fragments containing bacteria were performed in a specially designed chamber where the total count of aerobic bacteria originating from the skin flora was determined.

Estimation of total number of organisms (total viable cell count) compared with total viable particle number (CFU). The total number of microorganisms was estimated after the particles containing bacterial aggregates impacted on agar plates or trapped on filters had been dispersed. Experiments were run simultaneously with two Andersen samplers in environments with a high content of airborne bacteria. One Andersen sampler was handled as described above; the other was loaded with Exagar plates with a low concentration of agar (0.8%). After samling, the agar was cut up, mixed with physiological saline, and homogenized in a sterile Potter-Elheim homogenizer. The agar slurry was diluted, and 0.1-ml portions were inoculated onto the appropriate medium. The plates were incubated as described above, and the colonies were counted. This number was compared with the number of colony-forming units (CFU) estimated after incubating the agar plates directly after impaction.

Other experiments were run in the same environments with two filtration samplers. Filters from one sampler were placed directly onto the agar as described above. Filters from the other sampler were cut up and shaken in physiological saline with glass beads to break up the bacterial aggregates. Samples from serial dilutions of the fluid were inoculated onto agar plates. The bacterial counts were taken as the total number of microorganisms and compared with the number of CFU growing directly on the filters.

Fluids and agar plates were chilled to +10°C or less during transportation. In addition, all treatments of agar plates and filters were performed within 30 min after sampling to lessen the error caused by bacterial growth.

Experimental designs. (i) Aerosol experiments. These experiments were carried out in an aluminum exposure chamber with a volume of 37 liters (19). The bacterial suspension was aerosolized with a Collison spray (19), generating a known concentration of bacteria with more than 90% single-cell particles. The bacterial air samplers were placed inside the chamber and run simultaneously for 1 to 5 min. In some of these experiments the Andersen sampler was not used.

(ii) Field experiments. These experiments were performed in cotton mills and in a sewage plant. The samplers were placed roughly 1 m from the floor.

(iii) Skin fragment experiments. The sampling of bacteria carried on airborne skin fragments was performed in a chamber ventilated at the bottom through an absolute filter (Munktell AB) to make it bacteria-free. The air samplers were placed inside the chamber. A test person was asked to move around in the chamber for 5 min. Test persons were volunteers from the laboratory staff and medical students.

Calculations. The results are given as mean CFU per liter of air, and the standard deviation, $(SD)_{n-1}$, was calculated in each experiment for the different samplers used.

The P values in the statistical analysis are related to two-sided testing of the Student's t test. The paired t-test was also used.

RESULTS

Aerosol experiments. A total of 111 aerosol samples with *S. epidermidis* or *S. marcescens* were tested in five different experiments run on different days. Not all samplers were used in every experiment, although the slit sampler was always run.

With *S. epidermidis*, the Andersen sampler gave 15.7 ± 6.1 CFU per liter when all samplers were used simultaneously, which was significantly higher than all other samplers ($P < 0.001$). The slit sampler gave 10.1 ± 5.1 CFU per liter, which was significantly higher ($P < 0.001$) than the impinger value of 6.3 ± 4.9 and the membrane filter value of 6.1 ± 4.4.

With *S. marcescens*, the Andersen sampler value was 4.2 ± 1.2 CFU per liter, which was significantly higher than the values, in CFU per liter, obtained by the other samplers: 2.0 ± 0.6 with the slit sampler ($P < 0.001$, paired t-test), 3.2 ± 1.6 with the impinger ($P < 0.05$, paired t-test), and 0.8 ± 0.8 with gelatin filters ($P < 0.001$, paired t-test). The membrane filter value

TABLE 1. Results from experiments sampling for airborne skin fragments carrying viable bacteria

Sampler	No. tested	Bacterial count[a]
Andersen sampler I	53	100
Andersen sampler II	14	104 (22)
Slit sampler	22	78 (19)
Filter sampler, gelatin	45	66[b] (29)
Filter sampler, membrane	30	122 (47)

[a] The values represent the mean relative percentage of the Andersen sampler value. The numbers in parentheses are the standard deviations.

[b] $P < 0.001$ in relation to the Andersen sampler.

of 0.07 ± 0.08 CFU per liter was less than that of the gelatin filter, although the difference was not statistically significant.

Field experiments. Measurements in a cotton mill were performed with two Andersen samplers and a slit sampler ($n = 14$). The Andersen samplers gave 31.9 ± 9.3 and 34.9 ± 8.1 CFU per liter, which is significantly higher than the slit sampler value of 17.6 ± 8.7 CFU per liter ($P < 0.001$). The slit sampler value was 0.53 of the pooled Andersen sampler values. The impinger and filtration samplers were not used in the cotton mill.

In the sewage plant ($n = 20$), the Andersen sampler value (9.9 ± 4.1 CFU per liter) was significantly higher in both experiments than the bacterial count on the gelatin filters (0.3 ± 0.2; $P < 0.001$). The slit sampler value of 7.7 ± 2.9 CFU per liter was 78% of the Andersen sampler value. This difference was not statistically significant.

Skin fragment experiments. Table 1 shows results from the experiments sampling for skin fragments carrying bacteria. The number of airborne bacteria varied between 0.4 and 27.9 CFU per liter, as measured by the Andersen sampler, depending upon the test person used in the experiment. The slit sampler gave 0.78 of the Andersen sampler value, while the gelatin filter gave 0.66 and the membrane filter gave 1.2 times the value of the Andersen sampler. There were statistical differences (paired t-test) in sampling efficiency between the Andersen sampler and the gelatin filters ($P < 0.001$), between the slit sampler and gelatin filters ($P < 0.02$), and between the membrane filters and gelatin filters ($P < 0.001$).

Particle sizes. The mean size distribution of the particles containing viable bacteria from the different environments was measured by the Andersen sampler. In the aerosol experiments, 99% of the *S. epidermidis* and 98% of the *S. marcescens* were deposited on the last two stages, which sample particles of approximately 2 μm or less (1).

The majority of the particles containing viable gram-negative bacteria in the cotton mill fell within the size range of 2 to 6 μm (47%). In the sewage plant, the particles containing gram-negative bacteria were slightly larger: 62% were 5 μm or larger, and 30% were between 2 and 6 μm. Of the particles collected in the skin fragment experiments, 48% of those containing bacteria were 8.2 μm or larger.

Total number of organisms compared with total particle number. The results from the experiments in which the sampled aggregates of bacteria were broken up before plating are presented as the mean index of sampled and separated bacteria in relation to the CFU per liter value measured by the Andersen sampler at the same time (total number of viable organisms per total viable particle number). The bacterial counts after the aggregates had been dispersed were 13 times the Andersen sampler control count in the cotton mill, 24 times higher in the sewage plant, and 147 times higher in the skin fragment experiments. All values were significantly higher than the Andersen control ($P < 0.001$, paired t-test). When the filters were cut up and shaken, the counts in the skin fragment experiments were nine times higher with membrane filters and six times higher with gelatin filters than the counts for the respective control filters. The differences were not statistically significant.

DISCUSSION

The purpose of the present investigation was to evaluate several methods for determining the content of airborne bacteria in environments where they may cause medical problems. The methods used in this investigation have not previously been studied simultaneously in a comprehensive study.

The Andersen sampler gave the highest viable bacterial counts in all environments tested. The bacteria were counted according to the "positive hole" method (1) on all plates (13) and counts were corrected as suggested by Andersen (1). The "positive hole" method is a count of the jets that have delivered particles containing viable bacteria. The method preferred by Andersen (1), counting microcolonies under the microscope, was not performed due to difficulty in adjusting the incubation time to suit this method of calculation. In all experiments in this study, the Andersen sampler was used without the intake cone to lessen the cutoff of larger particles, which has been shown to be 50% for particles of a diameter larger than 12 μm (11, 13).

The slit sampler gave lower counts than the Andersen sampler in all environments tested. However, the difference was statistically signifi-

cant only in the aerosol experiments and the cotton mill experiment. This could be due to the difference in size of the particles carrying viable bacteria. In the aerosol experiments, the majority of the bacteria-carrying particles, as measured with the Andersen sampler, were 2 μm or less, and in cotton mills, they were 2 to 6 μm. On the contrary, in the sewage plant and particularly in the skin fragment experiments, the particle sizes were mainly 5 μm or larger.

According to Bourdillon et al. (3), the slit sampler is not efficient for trapping small particles. The Andersen sampler, on the other hand, is designed for the collection of particles down to 1 μm (1).

The impinger counts in the aerosol experiments were significantly lower than the Andersen sampler values. This is probably the case in experiments with aerosols where the bacteria are mainly single-cell particles and is in accordance with the findings of Andersen (1). In contrast, broth samplers tend to give higher bacterial counts in environments where bacteria are carried as aggregates, due mainly to the fact that bacterial clusters are broken up (7). In previous studies, broth samplers showed a higher coefficient of variability than the agar samplers, owing to the errors associated with plating out the samples (7). This was also the case in this investigation.

The efficiency of the filters varied between the environments tested. When sampling skin fragments carrying bacteria, the membrane filters gave 1.22 and the gelatin filters gave 0.66 of the Andersen sampler count. These results are contradictory to the findings of other authors. Fields et al. (8) found that membrane filters detected 79% of the bacterial count detected with a slit sampler. Hambraeus and Benediktsdóttir investigated the ability of filters to trap anaerobic airborne bacteria and found gelatin filters more efficient in sampling strict anaerobes (9). The reason for this is not known. In sewage plant and aerosol experiments, however, the gelatin filters gave less than 0.1 of the sampler value. The bacteria in these environments are freshly aerosolized and are susceptible to hydration. This may influence the capacity of the filters.

The properties of gelatin filters have been thoroughly investigated by Koller and Rotter (10, 18). The filtration efficiency of gelatin filters for all types of particles of 0.5 to 3 μm was shown to be 99.9%, as tested in room air with a Royco particle counter. However, the efficiency of trapping particles does not necessarily coincide with the sampling of viable bacteria. The bacterial yield was shown to decrease with filtration time. A 60-min filtration period gave only 55% of the yield of a 1-min period. The sampling time in this study was 1 to 5 min to lessen the desiccation of the sampled bacteria, but still, the filters were not very efficient.

The difference in efficiency between gelatin filters and membrane filters in the skin fragment experiments could be due to a difference in trapping efficiency or to a variation in ability to support the growth on the filters. Koller and Rotter (10), however, found both types of filters about equally efficient with short filtration times. The experiments in which the total viable cell count was estimated showed a rising index value with increasing particle size when using the Andersen sampler. A mean of 13 bacteria was carried per particle in the cotton mill, and a mean of 147 bacteria was carried on skin fragments. The index value obtained when using filters was much less. The skin flora has been shown to consist of microcolonies with a mean of 124 or more aerobic bacteria (16). Studies in hospital environments of *Staphylococcus aureus* on airborne particles have shown an average of four viable organisms per particle with a 13-μm diameter (12). Bacteria on skin scales are usually found in the equivalent diameter range of 4 to 20 μm (15). May and Pomeroy (14) found a mean of 4.6 viable counts per viable particle, measured with a cyclone sampler and a slit sampler, respectively. This is, however, much lower than the values found in this study. The method of homogenizing the bacterial aggregates may be more efficient than the cyclone sampler in separating the bacteria. May and Pomeroy (14) found a rather large scatter in the results. This was also the case in this investigation.

In summary, of the samplers tested, the Andersen sampler showed the highest sampling efficiency in all environments tested, regardless of whether the majority of the particles carrying bacteria were 1 to 2 μm in size, or mainly 5 μm or larger. The slit sampler gave significantly lower counts in areas with smaller particles, but not in sewage and skin fragment experiments, where the particle sizes are larger. The filters were satisfactory in skin fragment experiments only.

LITERATURE CITED

1. **Andersen, A. A.** 1958. New sampler for the collection, sizing, and enumeration of viable airborne particles. J. Bacteriol. **76**:471–484.
2. **Bourdillon, R. B., O. M. Lidwell, and J. C. Thomas.** 1941. A slitsampler for collection and counting airborne bacteria. J. Hyg. **41**:197–224.
3. **Bourdillon, R. B., O. M. Lidwell, and J. C. Thomas.** 1948. A large slitsampler for air containing few bacteria. Med. Res. Counc. G.B. Spec. Rep. Ser. **262**:19–22.
4. **Charnley, J.** 1972. Postoperative infection after total hip replacement with special reference to air contamination in the operating room. Clin. Orthop. **87**:167–187.
5. **Cinkotai, F. F., M. G. Lockwood, and R. Rylander.** 1977. Airborne microorganisms and prevalence of byssinotic symptoms in cotton mills. Am. Ind. Hyg. Assoc. J. **38**:554–559.

6. **Cinkotai, F. F., and C. J. Whitaker.** 1978. Airborne microorganisms and prevalence of byssinotic symptoms in 21 cotton spinning mills in Lancashire. Ann. Occup. Hyg. **21**:239–250.
7. **DuBuy, H. G., and A. Hollaender.** 1945. Sampling devices. Am. J. Med. Sci. **209**:172–177.
8. **Fields, N. D., G. S. Oxborrow, J. R. Puleo, and C. M. Herring.** 1974. Evaluation of membrane filter field monitors for microbiological air sampling. Appl. Microbiol. **27**:517–520.
9. **Hambraeus, A., and E. Benediktsdóttir.** 1980. Airborne nonsporeforming anaerobic bacteria. J. Hyg. **84**:181–189.
10. **Koller, W., and M. Rotter.** 1974. Weitere Untersuchungen über die Eignung von Gelatinefiltern zur Sammlung von Luftkeimen. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. B **159**:546–559.
11. **Lidwell, O. M., and W. C. Noble.** 1965. A modification of the Andersen sampler for use in occupied environments. J. Appl. Bacteriol. **28**:280–282.
12. **Lidwell, O. M., W. C. Noble, and G. W. Dolphin.** 1959. The use of radiation to estimate the numbers of microorganisms in airborne particles. J. Hyg. **57**:299–308.
13. **May, K. R.** 1964. Calibration of a modified Andersen bacterial aerosol sampler. Appl. Microbiol. **12**:37–43.
14. **May, K. R., and N. P. Pomeroy.** 1973. Bacterial dispersion from the body surface, p. 426–432. *In* J. F. Ph. Hers and K. C. Winkler (ed.), Airborne transmission and airborne infection. VIth International Symposium on Aerobiology. Oosthoek Publishing Co., Utrecht, The Netherlands.
15. **Noble, W. C., O. M. Lidwell, and D. Kingston.** 1963. The size distribution of airborne particles carrying microorganisms. J. Hyg. **61**:385–391.
16. **Noble, W. C., and D. A. Somerville.** 1974. Microbiology of human skin, p. 60. *In* Major problems in dermatology, vol. 2. The W. B. Saunders Co. Ltd, London.
17. **Phillips, G. B., and R. S. Runkle.** 1973. Biomedical applications of laminar airflow. CRC Press, Boca Raton, Fla.
18. **Rotter, M., and W. Koller.** 1973. Sammlung von Luftkeimen mit Gelatinefiltern. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. B **157**:257–270.
19. **Rylander, R.** 1968. Pulmonary defence mechanisms to airborne bacteria. Acta Physiol. Scand. Suppl. **306**:1–89.
20. **Rylander, R., K. Andersson, L. Belin, G. Berglund, R. Bergström, L. Hanson, M. Lundholm, and I. Mattsby.** 1977. Studies on humans exposed to airborne sewage sludge. Schweiz. Med. Wochenschr. **107**:182–184.
21. **Rylander, R., and M. Lundholm.** 1978. Bacterial contamination of cotton and cotton dust and effects on the lung. Br. J. Ind. Med. **35**:204–207.